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AGE AND THE CHEMICAL CONSTITUTION OF NORMAL HUMAN DERMIS*

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ABSTRACT

Samples of normal dermis were collected from seven women and six men ranging in age from 17 to 81 years. The water content increased with age while the total lipid remained unchanged. The tissue was separated into fractions representing 'ground substance' (isotonic saline extract), cells (tryptic digest), and collagen (gelatin). Each fraction was analyzed for nitrogen, hydroxyproline, hexosamine and hexuronic acid. The amount of the saline extract changed little with age. The amount of both the trypsin and gelatin fractions decreased with age. None of the measured components could be related to the sex of the donor.

The proteins of the isotonic saline extract were examined by cellulose acetate, disc and immunoelectrophoresis. Qualitatively, all fractions resembled the plasma proteins. The relative proportions of the proteins corresponded closely to those in the plasma. The hemaglobin content of the extract was less than one percent that expected if the proteins were present in whole blood.

Most of the hyaluronate of the dermis was found in the ground substance. Dermatan sulfate predominated in the cellular and collagenous fractions. The age and sex of the donor could not be related to the concentration of any fraction.

The residue resembled pure elastin in its appearance under the light microscope after staining with hematoxylin and eosin, Heidenhain's azan or aldehyde fuchsin; its ultrastructure after sonication; its nitrogen and hydroxyproline content; and its amino acid analysis. The amount increased three-fold between 20 and 80 years of age.

The chemical composition of normal human dermis has been uncertain because the available data have been difficult to interpret. The specimens analyzed have frequently included unknown and variable quantities of epidermis, hair follicles, accessory glands, adipose tissue and loose subcutaneous connective tissue; few attempts have been made to separate quantitatively the major anatomical components—cells, fibers and ground substance. Also, the analyses used to measure the chemical constituents have often not been specific. Recent work has overcome some of these objections (1–5). The development of a quantitative method for the separation of the major anatomical components of rat skin (6) has permitted a study of normal human dermis which minimized many of these difficulties. Preliminary accounts of some aspects of this work have been published (7, 8).

MATERIALS AND METHODS

Specimens. Well nourished subjects free of chronic disease were sought. These criteria were approximated by the subjects described in Table I. Specimens were collected 6 to 40 hours *post mortem;* the average delay was 19 hours. A specimen approximately 25 cm long and 6 cm wide was cut from the skin of the chest just posterior to the midline, where the skin is relatively free of hair and accessory glands. The adipose and loose subcutaneous tissues were carefully dissected from the inner surface. The epidermis and the outer layer of the dermis were then removed with a Stryker (T.M.) dermatome set at 0.01 in. The dermis was minced with scissors and passed through a Latapie tissue grinder. Duplicate 2.5 to 4.5 g portions were weighed into 90 ml Foust centrifuge tubes and freeze-dried. Specimens were stored at −80° until extraction.

Extraction. The dermis was fractionated by the procedure described earlier (6), modified principally by the substitution of the volatile 0.2 *M* triethylamine bicarbonate, pH 8.0, for the sodium bicarbonate buffer used in the treatment with trypsin. The residue was dried *in vacuo* over anh. calcium chloride before weighing.

Histology. Samples were fixed in formol-calcium acetate, embedded in paraffin, sectioned at 5 μm, and stained with either hematoxylin and eosin, Heidenhain's azan procedure for collagen or Gomori's aldehyde fuchsin-alcian blue for elastin (9).

Analyses of extracts. Saline extract equivalent to 0.80 g fresh dermis was dialyzed, freeze-dried and dispersed in 10 ml water. Tryptic digest representing 1.5 g fresh dermis was freeze-dried and dispersed in 6 ml water. The gelatin extract was analyzed as prepared at a dilution of 20 ml per g dermis. *Nitrogen* was estimated by a micro-Kjeldahl-Nesslerization procedure (10). Samples for *hexosamine* were hydrolyzed in 4 *N* HCl *in vacuo* for 16 hours at 100°, dried *in vacuo* at room temperature, purified on Dowex-50(H^+) columns and analyzed by the Elson-Morgan reaction (11). *Hydroxyproline* was estimated by Procedure II of Prockop and Udenfriend (12), using the humin precipitant. *Hexuronic acid* was estimated by the carbazole-borosulfuric acid reaction (13).

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Proteins of the saline extract. A 25-ml portion of each saline extract was clarified, concentrated and analyzed by procedures described elsewhere (8).

Glycosaminoglycans (GG). The protein of the gelatin fraction was reduced by digestion of a portion representing 2.5 to 4.0 g fresh dermis with 5 μg *Streptomyces griseus* protease (Pronase, Calbiochem) per ml 0.1 *M* tris(hydroxymethyl)aminomethane (TRIS) hydrochloride, pH 8.0, at 60° for 16 hours, followed by dialysis and freeze-drying of the digest. Portions of tryptic digest, representing one to three g fresh dermis, were freeze-dried. The GG from these and the saline fraction were isolated, fractionated and characterized by published procedures (8).

A more critical identification of the GG was undertaken on fractions isolated by a scaled-up version of the procedures referred to above (8), but using 83 g of dermis collected from a leg amputated because of circulatory insufficiency leading to gangrene; grossly-abnormal skin was excluded from the specimen. In addition to the analyses performed on the smaller specimens, hexuronic acid was identified by paper chromatography after hydrolysis in *N* HCl at 100° for 4 hours (14, 15), hexuronic acid (13) and nitrogen (10) were estimated, and the homogeneity of the fractions examined by paper electrophoresis (16, 17).

Examination of the residue. A 1 to 2 mg portion was hydrolyzed in 6 *N* HCl *in vacuo* overnight in an autoclave at 20 lb per sq in. The hydrolyzate was dried and analyzed for nitrogen (10) and hydroxyproline (12). Amino acid analyses were performed on six selected samples (18), by Dr. L. B. Sandberg, Department of Surgery, University of Utah Medical Center, Salt Lake City. Other portions were soaked six days at 4° in water, homogenized at top speed for 15 minutes at 4° in 10 ml water (Sorvall Omnimixer, 50-ml cup). The heavy particles were allowed to sediment and the supernate was sonicated 15 min at 4° and 20 kHz at 60% maximum power (Biosonik probe). A drop of the suspension was placed on a celloidin-coated grid and the dry residue was shadowed with carbon and platinum. The preparation was examined in an electron microscope.

TABLE I

Patients included in this investigation

Spec. no.	Age (yr)	Sex	Principal cause of death
1	53	M	Myocardial infarction during the implantation of a prosthetic valve for aortic stenosis.
2	17	F	Acute viral encephalitis (one-week course).
3	25	F	Fractured skull with brain damage.
4	64	M	Myocardial infarction.
5	29	M	Glioblastoma.
6	41	F	Fractured skull with brain laceration.
7	64	F	Multiple cerebral thrombi following surgery.
8	32	M	Pulmonary hypertension leading to right heart failure after repair of ventricular septal defect.
9	75	F	Multiple embolic infarctions in kidney, bowel and spleen.
10	70	M	Cerebrovascular accident.
11	41	M	Tachycardia leading to acute tubular necrosis following implantation of prosthetic valve for aortic stenosis.
12	80	F	Cerebrovascular hemorrhage.
13	81	F	Coronary thrombosis.

Statistical analyses. The data were analyzed using methods described by Snedecor (19). Each set of data were examined to ascertain the effects of sex and age. Individual differences were compared with analytical uncertainty using an analysis of variance. The variance due to individual differences was examined for differences between men and women. The significance of trends with age was tested by calculation of a coefficient of correlation and, if this differed from zero ($P < 0.05$), the linear regression equation and deviation from regression were also calculated. The latter statistic was taken as a measure of the range of values expected in individuals of the same age. If the correlation was not significant, a mean and standard deviation were calculated.

RESULTS

Histology. The appearance of the sections, both before and during the extraction process, was quite uniform. The skin contained relatively few hair follicles, sebaceous and sweat glands. Since the epidermis was relatively free of intercapillary pegs, the dermatome removed the epidermis quite completely with the loose subpapillary layer of the dermis attached (Fig. 1A). Because the skin was supported by a dense sponge-rubber sheet while the dermatome was used, the thickness of tissue removed was approximately double the nominal setting. The tissue analyzed comprised almost entirely the dense connective tissue from the deep layers of the dermis with an occasional hair follicle, sweat and sebaceous gland (Fig. 1B). Occasionally, fragments of epidermis and of adipose tissue were seen. The collagen appeared consistently as bundles of fibers with a fine, interlacing network of elastic fibers.

Freeze-drying and extraction of the lipid did not materially alter the appearance of the tissue, apart from the disorganization associated with mincing and the shrinkage of the cells after petroleum ether. During the sequential treatments with isotonic saline and trypsin, the bundles of collagen fibers separated into a loose network of individual fibers. In many specimens, the expansion was complete after the saline extraction; in others, the process was not complete until the use of trypsin. This effect was unrelated to the age and sex of the individual; indeed, duplicate specimens from an individual often behaved differently in this respect. Cells were uniformly present and of normal appearance after the saline treatment, although the expansion of the tissue caused them to appear sparsely distributed. Trypsin consistently removed any evidence of cellular content except, in an occasional specimen, an ill-defined basophilic blob suggestive of an epidermal residue was seen. These observations are illus-

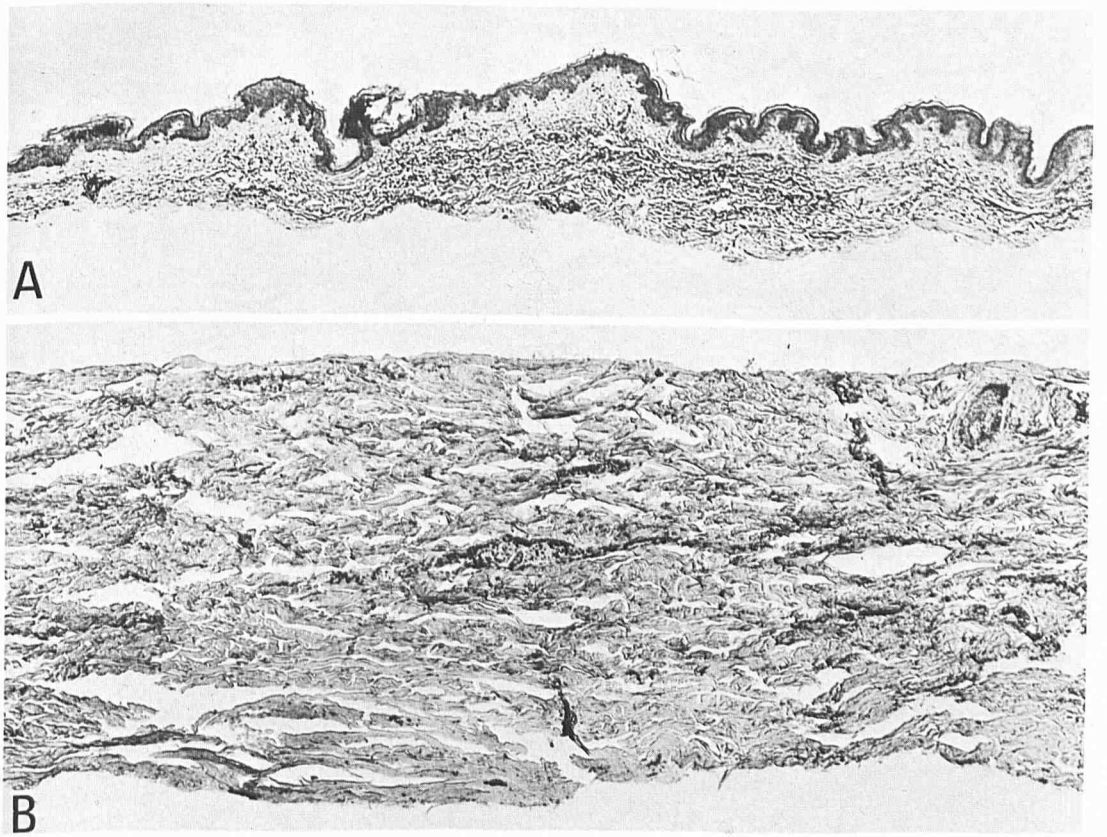

FIG. 1. *Histological appearance of specimens* × 48, H.&E. *A*. Epidermis and subpapillary layer of dermis. *B*. Dermis, used for extraction and analysis.

trated in Figure 2A, B, C. No evidence of collagenous fibers was seen after autoclaving. The elastic fibers remained throughout the entire procedure, showing only a tendency to fragment and swell with successive stages of treatment. The residue was singularly uniform in appearance—dense, curled, refractile fibers, faintly eosinophilic, resistant to the azan stain and strongly reactive with aldehyde-fuchsin (Fig. 3).

Water and fat. As shown in Figure 4, the water content of the dermis increased with age ($P \sim 0.01$), whereas the lipid content remained constant ($P > 0.05$). No difference between the sexes was found in either water or fat content.

Composition of the fractions. The nitrogen, hydroxyproline, hexosamine and hexuronic acid contents of the saline, trypsin and gelatin fractions are shown in Figure 5. Nitrogen was used to assess the total protein of the fraction; hydroxyproline, the collagen; hexosamine, the glycosaminoglycans and glycoproteins; and hexuronic acid, the glycosaminoglycans. Hexoses, as well as hexuronic acids, are chromogens with the carbazole-borosulfuric acid reaction (13), making difficult the interpretation of results, since glycoproteins would be expected to contribute to the measurement. The techniques were sufficiently reproducible to permit the detection of differences between individuals (Table II and Fig. 5). No differences between men and women could be demonstrated ($P > 0.05$). The analysis of the trends with age is shown in Table II and Figure 5. The nitrogen, hexosamine and hydroxyproline of the saline extract did not change with age. The progressive decline in the hexuronic acid chromogen has not been explained. The components of the trypsin fraction declined with age. The nitrogen, hydroxyproline and hexosamine of the gelatin fraction each declined progressively with age.

Proteins of the Saline Extract†

The analyses of the saline extract revealed the principal component to be nitrogen, suggesting that protein was a major constituent. The concentrated extract was examined by cellulose acetate electrophoresis (see Fig. 6). The process of concentration tended to produce a somewhat irregular pattern with reference serum, an effect which was exaggerated with the saline extract. This phenomenon is believed due to contamination of the concentrate with small amounts of polyvinylpyrrolidone. The osmotic dialysis used to reconcentrate the serum produced diffuse bands in the

† Preliminary reports of the results described in this section have been published (7, 8).

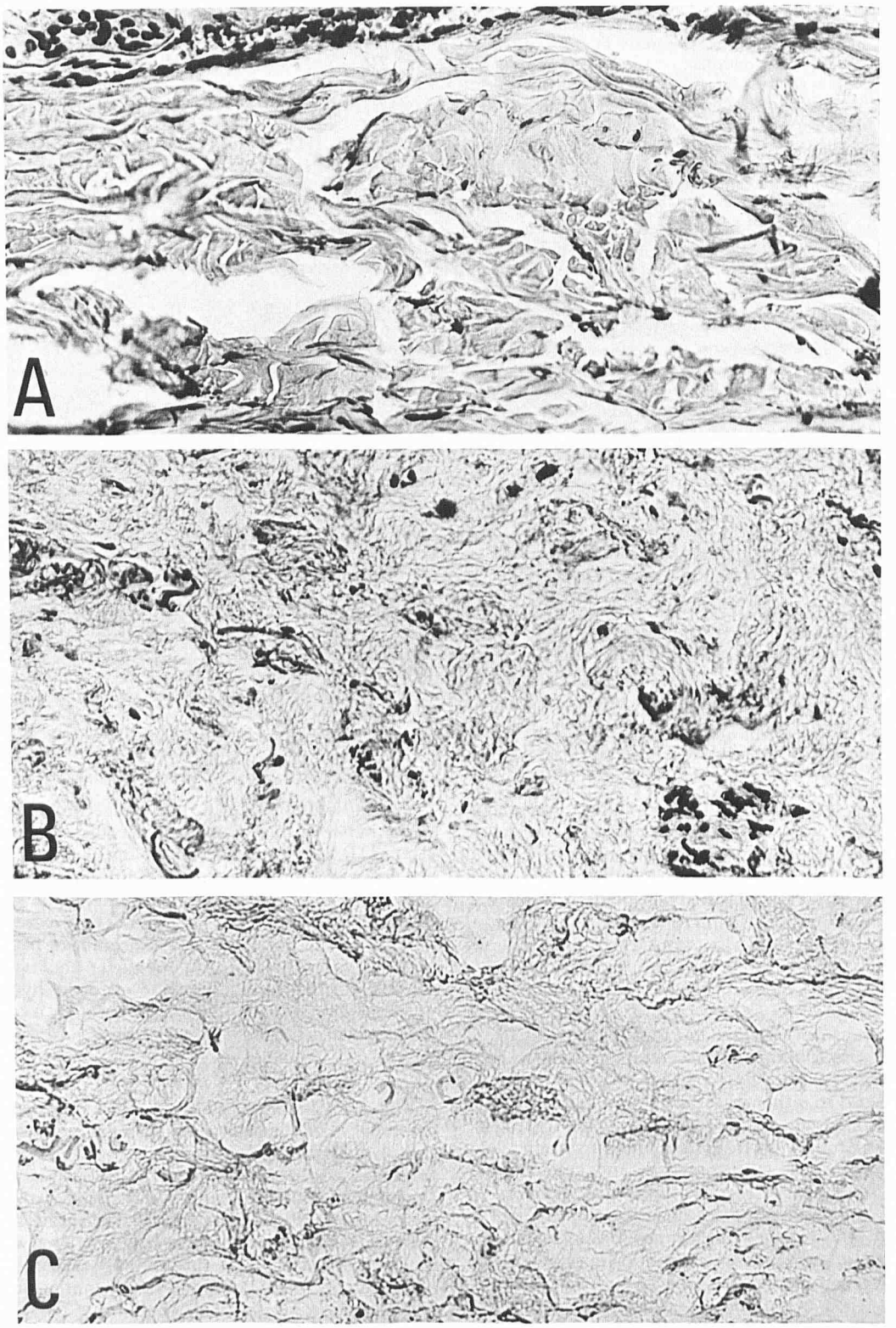

FIG. 2. *Changes in histological appearance of dermis during extraction.* × 340, H.&E. *A*. Fresh dermis. *B*. After extraction with isotonic saline. *C*. After treatment with trypsin.

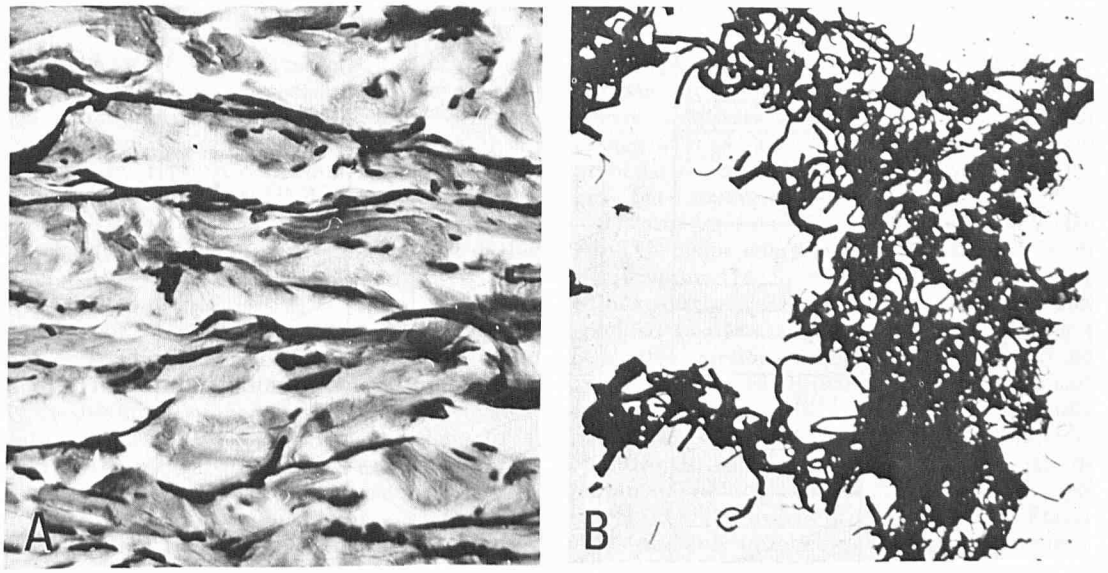

FIG. 3. *Human dermis* × 340, aldehyde-fuchsin. *A*. Fresh dermis. *B*. Residue after saline extraction, tryptic digestion, and autoclaving.

alpha region and sharpened the bands in the gamma region. The saline extract showed bands in the albumin, beta and gamma regions, with diffuse staining between the bands. Photoelectric densitometry of the strips gave the results shown in Table III. The untreated reference serum gave values in the stated range. Concentration resulted in a net loss from the gamma region with a proportionate increase in the alpha bands. If allowance is made for this adverse effect of the process of concentration, the values for the saline extract fall within the range expected for normal individuals (20). The range of values observed did not exceed greatly the analytical error of the procedure, suggesting that the latter was a major influence on the distribution of the values about the means. Apart from the denaturation resulting from their concentration, the proteins of the saline extract appeared to resemble those of the plasma in mobility and relative composition.

The proteins were resolved in greater detail by the use of disc electrophoresis in polyacrylamide gel. The diluted and concentrated reference serum yielded a pattern closely resembling that of untreated serum, the most consistent difference being the diminished resolution of the haptoglobins (see Fig. 7). Many of the same bands were seen in the 13 replicate concentrated saline extracts studied: pre-albumin, albumin, ceruloplasmin, transferrin, α2-macroglobulin, β-lipoprotein and immunoglobulin-G. The haptoglobins were seen in only 10 of the 25 specimens. Thus, disc electrophoresis also supported the identity between the proteins of the saline extract and those of the plasma. The only unusual finding consistently seen was a prominent band with the mobility of ceruloplasmin, visible in Figure 7.

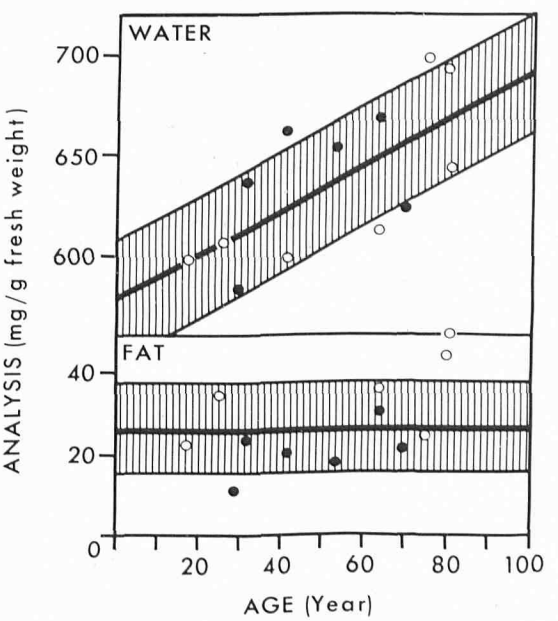

FIG. 4. *The water and fat content* of normal dermis from men (●) and women (○) of differing ages.

The identity of the dermal with the plasma proteins was further examined by immunoelectrophoresis (see Fig. 8). After dilution and reconcentration, the pattern matched very closely that seen in the control, the main difference being the presence of some additional bands near the

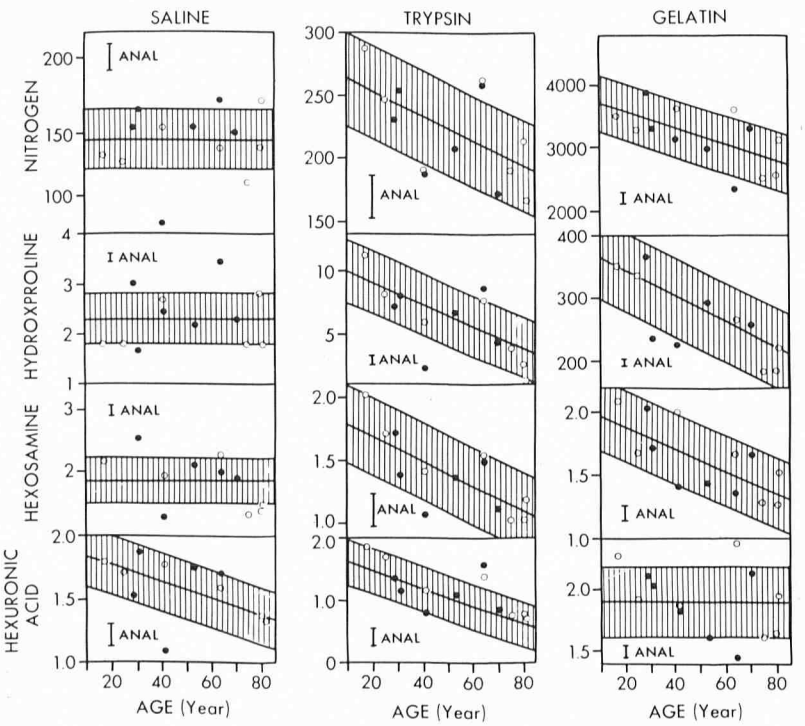

FIG. 5. *The analyses of the saline, trypsin and gelatin fractions* of human dermis from men (●) and women (○), expressed as μmole per gm fresh weight. The analytical error (ANAL) is shown on each section of the figure.

TABLE II

The composition of the dermis fractions: statistical analyses

An analysis of variance was performed as described by Snedecor (19); the individual variation was corrected for the contribution of the analysis. The correlation of the analysis with age was calculated and, if significant, the equation of the regression line determined.

Fraction	Analysis	Standard deviation* (μmole/g fresh wt.) associated with		Significance of changes with age†
		Analysis	Individuals	
Saline	Nitrogen	11	24**	$P > 0.1$
	Hydroxyproline	0.36	0.50**	$P > 0.1$
	Hexosamine	0.096	0.36**	$P > 0.1$
	Hexuronic acid	0.081	0.26**	$P \sim 0.05$
Trypsin	Nitrogen	16	53**	$P \sim 0.05$
	Hydroxyproline	0.36	2.5**	$P < 0.01$
	Hexosamine	0.13	0.27**	$P < 0.01$
	Hexuronic acid	0.13	0.35**	$P \sim 0.01$
Gelatin	Nitrogen ($\times 10^{-3}$)	0.096	0.46**	$P < 0.05$
	Hydroxyproline	5.2	63**	$P < 0.01$
	Hexosamine	0.06	0.28**	$P \sim 0.01$
	Hexuronic acid	0.056	0.28**	$P > 0.1$

* Calculated by analyses of variance.

** $P < 0.01$, for F-test of individual variation vs. analytical error.

† As estimated by a t-test of the assumption that the coefficient of correlation equals zero.

origin. Since these were seen in the undiluted reference serum, their absence in the control seems likely to be due to their lower concentration. The patterns for the concentrated saline extract resembled closely those of the diluted reference serum. In addition to several minor bands which could not be clearly identified, pre-albumin, albumin, transferrin and the immunoglobulins-G, -M and -A were consistently seen in the ten specimens examined. Thus, by each of the three methods used, the proteins of the saline extract corresponded to those of the plasma.

The mere presence of plasma proteins in the extract of dermis is not sufficient to demonstrate the extravascular location of the proteins. Definitive studies of this problem have been left for later work. However, the portion of the protein originating from within the vascular system was estimated by measurement of the hemoglobin content of the extract. The values ranged from 0.06 to 1.23 mg per g fresh dermis, with a mean of 0.45 ± 0.51 (S.D.). At a typical hemoglobin concentration of 15 g per 100 ml of blood, i.e. 0.15 mg per μl of blood, the average hemoglobin value suggests a blood content of 3 μl per g fresh dermis. On the other hand, if a typical plasma protein concentration of 6.3 g per 100 ml is assumed and a hematocrit of 50% the 12.6 mg of protein per g fresh dermis corresponded to a blood content of 400 μl per g of tissue. Much of the protein of the saline extract must have occurred extravascularly. The plasma protein content of the dermises corresponded to one-fifth that of an equal weight of plasma.

Glycosaminoglycans (GG)

Saline Extract. The polysaccharides of the saline fraction of each specimen of skin were isolated, fractionated and characterized with the results shown in Table IV. The analytical error, calculated by analysis of variance from duplicate samples, was sufficiently small, particularly for the large fractions, that individual differences were clearly apparent. A preliminary report of the data for the saline fraction has appeared previously (8).

The major fraction of the saline extract was Fraction I. The carbazole sulfuric-to-hexosamine ratio of 1.18 and the finding of glucosamine as the only hexosamine support its identity as hyaluronate. Because of the lower yield, the characterization of the other two fractions was more difficult. In every specimen of reasonable bulk, galactosamine was found on hexosamine chromatography. Fraction IIA resembled dermatan sulfate; the depressed carbazole sulfuric-to-hexosamine ratio suggested that this fraction was mainly an iduronic acid-containing moiety, although the average value of the ratio was high enough to suggest that some glucuronic acid was present as well (21). The ratio for Fraction IIB was close to that expected for a glucuronic acid-containing polysaccharide but the finding of glucosamine in nearly all specimens indicated that in addition to chondroitin sulfate, hyaluronate, keratan sulfate or heparitin sulfate may have been present; all have been reported to occur in skin (22).

Trypsin fraction. The results are given in Table IV. The major component of the trypsin fraction was Fraction IIA, for which the carbazole sulfuric-to-hexosamine ratio and galactosamine corresponded to dermatan sulfate. The yields for the other two fractions were sufficiently low to make accurate analyses difficult. However, Fraction I corresponded generally to the expected findings for traces of hyaluronate and Fraction IIB to chondroitin sulphate contaminated with dermatan sulfate and a glucosamine-containing polysaccharide. The apparently high ratio for Fraction I was probably unreliable due to analyses close to the limits of sensitivity of the method.

Gelatin fraction. These data also are given in Table IV. Because no hyaluronate was noted on electrophoresis, the fractionation on AG-1 was omitted for one-half the gelatin fractions. The subsequent finding of glucosamine in Fraction IIB

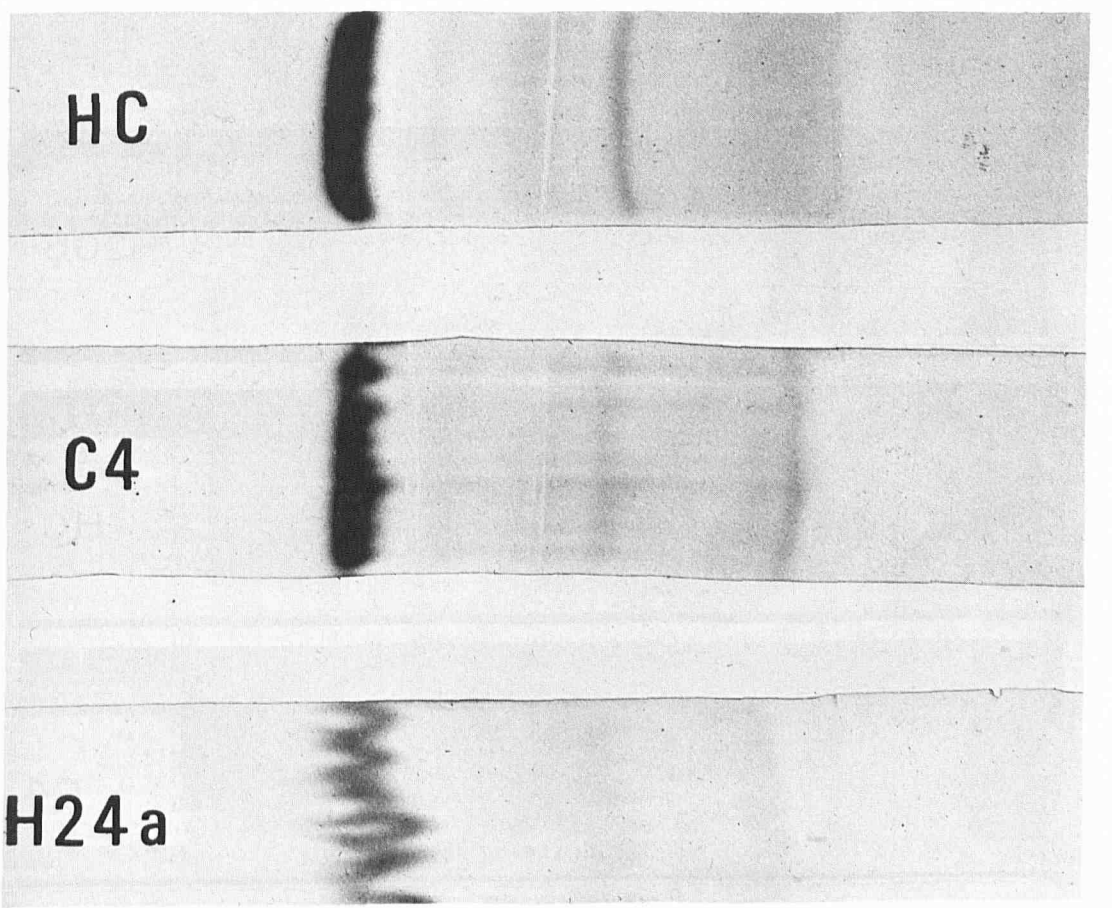

FIG. 6. *Cellulose acetate electrophoresis.* HC—untreated reference serum; C4—diluted and reconcentrated reference serum; H24a—a typical concentrated saline extract of dermis.

TABLE III

Electrophoresis of proteins on cellulose acetate†

Diluted and reconcentrated reference serum was compared with untreated reference serum. The effect of the treatment was assessed by an analysis of variance, from which the analytical error was also derived. The values under saline extract represent the mean and standard deviations obtained from separate specimens of dermis, the replicate values for each dermis having been averaged prior to calculation.

Fraction	Reference serum				Saline extract (Mean ± S.D.)
	Stated values	Untreated	Diluted and reconcentrated	Anal. error	
Albumin	56–64	63.2	62.2	3.0	67.6 ± 5.0
Alpha-1	2–4	2.7	5.2***	1.0	14.3 ± 3.0
Alpha-2	6–10	8.4	9.4	1.5	
Beta	8–14	10.8	10.6	1.2	10.9 ± 2.0
Gamma	15–21	15.6	11.8*	2.0	8.0 ± 3.0
No. of Specimens Tested		5	4		13

† All values given are relative concentrations, i.e. percent of total protein.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

suggested that this fraction might have been contaminated with hyaluronate. Accordingly, the AG-1 fractionation was included for the remainder of the specimens. The major fraction was IIA; its carbazole sulfuric-to-hexosamine ratio and galactosamine content were that expected for dermatan sulfate. Fraction IIB gave the results to be expected of a chondroitin sulfate, contrary to the findings for the saline and trypsin fraction. The yield of Fraction I was too small to permit critical analysis but corresponded to that expected for traces of hyaluronate.

Yields. The individual yields of each fraction are illustrated in Figure 9. These and a variety of combinations of fractions were examined carefully for trends which could be related to the age or the sex of the individual. No such trend could be demonstrated despite analytical errors sufficiently small to reveal individual differences.

Homogeneity and identity of polysaccharide fractions. The small yields had made difficult the accurate characterization of the polysaccharides isolated from the various fractions of the small specimens of dermis. To confirm the general impressions gained from the individual samples, a large specimen of dermis was processed through a scaled-up version of the isolation procedure. Each of the resulting nine polysaccharide fractions was

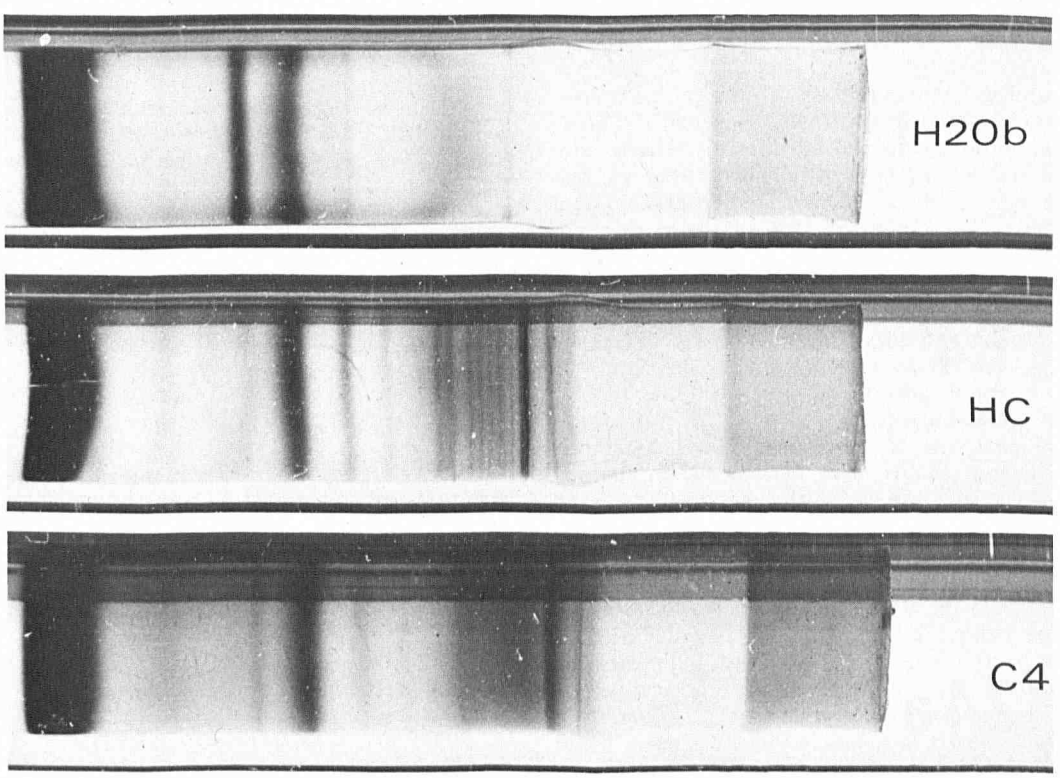

FIG. 7. *Polyacrylamide disc electrophoresis.* HC—untreated reference serum; C4—reference serum, diluted 100-fold with 0.15 *M* NaCl and re-concentrated; H20b—a typical concentrated saline extract.

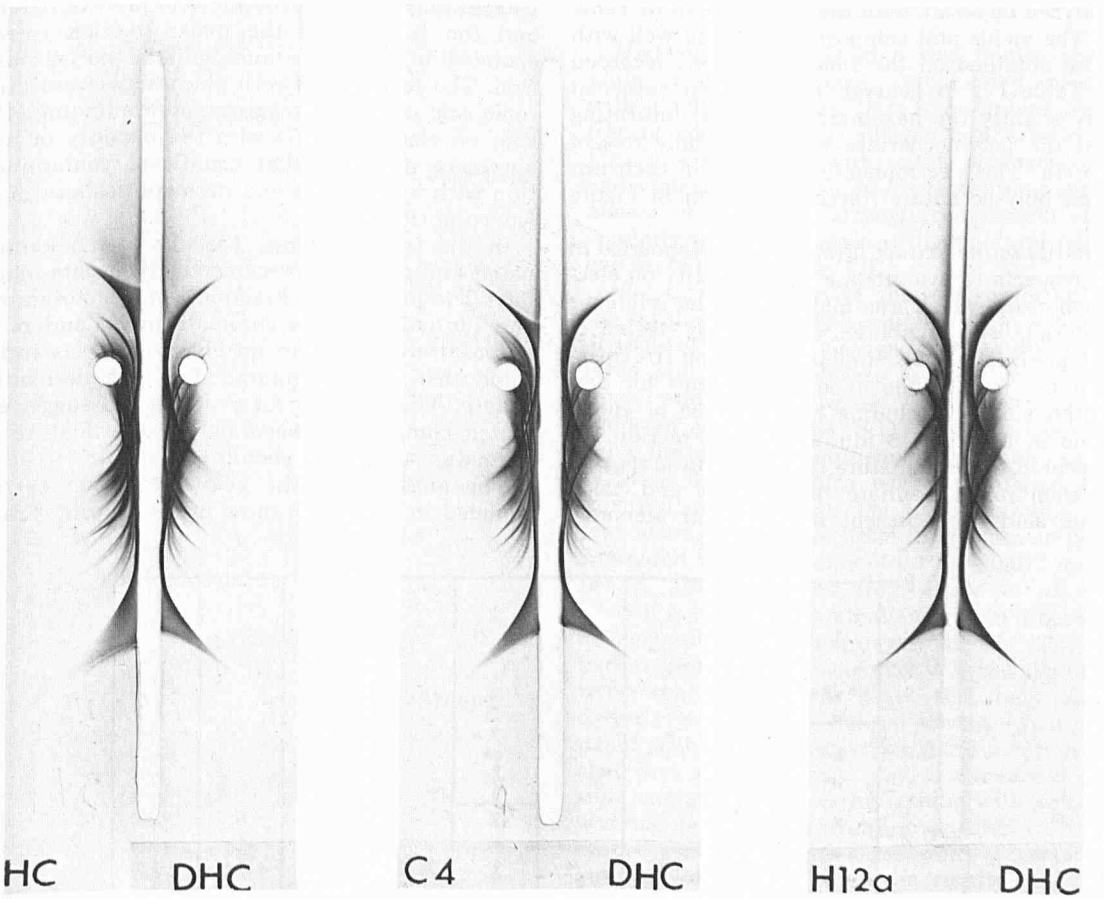

FIG. 8. *Immunoelectrophoresis.* Goat antihuman serum was used as antibody. DHC—reference serum, diluted to a protein concentration close to that of the concentrated saline extracts; HC—undiluted reference serum; C4—reference serum, diluted 100-fold with 0.15 *M* NaCl and reconcentrated; H12a—concentrated saline extract.

TABLE IV

The yields and characterization of dermal glycosaminoglycan fractions

Fraction of dermis	Polysaccharide fraction	Yield (μmole hexosamine/g fresh wt.)			Analyses			
		Mean	Indiv.* diff.	Anal.* error	Carb.: H_2SO_4 / hexosamine	Hexosamine		
						No.	$GlcNH_2$	$GalNH_2$
Saline	I	0.46	0.24	0.06	1.18 ± 0.17(24)	25	25	0
	IIA	0.08	0.02	0.06	0.61 ± 0.24(22)	23	1(tr)	22
	IIB	0.09	0.03	0.02	0.89 ± 0.25(23)	23	22	22
	Total	0.62	0.29	0.09				
Trypsin	I	0.04	0.02	0.01	2.43 ± 1.88(24)	22	21	0†
	IIA	0.27	0.15	0.05	0.54 ± 0.33(24)	24	1(tr)	24†
	IIB	0.10	0.03	0.02	0.65 ± 0.19(24)	25	10	24
	Total	0.42	0.23	0.07				
Gelatin	IIA**	0.20	0.09	—	(see below)	13	4	13
	IIB**	0.08	0.03	—	(see below)	13	11	12
	I	0.02	0.00	—	2.79 ± 0.86(11)	13	12	0
	IIA	0.12	0.06	—	0.56 ± 0.15(26)	13	0	13
	IIB	0.06	0.02	—	0.82 ± 0.38(26)	13	(tr)	13
	Total	0.24	0.19	0.06				
Total	(23)	1.25	0.44	0.14				

* Standard deviations, as determined by analyses of variance.

† Traces of galactose present.

** Fraction I was not separated in these specimens.

analyzed in detail, with the results given in Table V. The yields and compositions agreed well with those obtained on the small specimens recorded in Table IV. In general, the nitrogen exceeded only slightly the hexosamine content, indicating that the polysaccharides were essentially free of protein. The electrophoretic analysis of each isolated polysaccharide fraction is shown in Figure 10.

In the *saline* extract, Fraction I corresponded in all respects to hyaluronate. The mobility on electrophoresis, which was higher than the reference material, was attributed to its lower degree of polymerization (17). Similarly, Fraction IIA corresponded well to the expected findings for dermatan sulfate, including the presence of glucuronic in addition to iduronic acid. Fraction IIB appeared to be a mixture comprised in large part of chondroitin 4-sulfate: hexosamine and hexuronic acid were present in equimolar amounts; galactosamine and glucuronic acid predominated; and the mobility of the major fraction corresponded to that of a monosulfated polysaccharide. The presence of both glucosamine and iduronic acid on chromatograms and the finding of a spot on electrophoresis with the mobility of hyaluronate indicated that significant contamination with hyaluronate and dermatan sulfate may have occurred.

In the *trypsin* digest, Fraction IIA predominated and clearly corresponded to dermatan sulfate. The presence in Fraction I of galactosamine and iduronic acid on chromatography and of a monosulfated fraction on electrophoresis indicated that it was contaminated with dermatan sulfate. The analyses for Fraction IIB suggested that it comprised either a hybrid or a mixture of dermatan sulfate and chondroitin sulfate.

The analyses of the *gelatin* fraction corresponded in general to those of the trypsin. Frac-

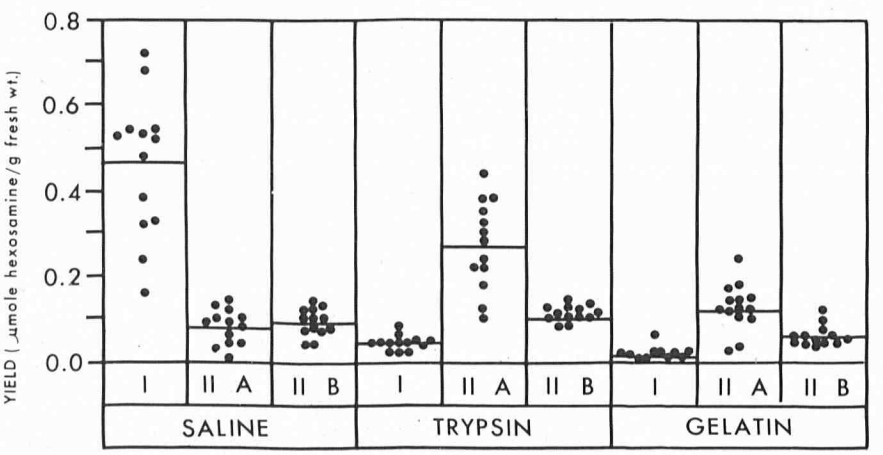

FIG. 9. *The yield of the various fractions of the glycosaminoglycans of human dermis.*

TABLE V

*Detailed analyses of dermal glycosaminoglycans**

Analysis	Extract and polysaccharide fraction								
	Saline			Trypsin			Gelatin		
	I	IIA	IIB	I	IIA	IIB	I	IIA	IIB
Hexosamine									
(μmole)	53.6	9.1	8.4	6.3	25.0	4.7	2.0	7.8	1.9
(μmole/g. fresh wt.)	0.44	0.08	0.07	0.05	0.21	0.04	0.02	0.06	0.02
chromatography	$glcNH_2$	$galNH_2$	$glcNH_2$ $galNH_2$	$glcNH_2$ $galNH_2\pm$	$galNH_2$	$galNH_2$	$glcNH_2$	$galNH_2$	$galNH_2$
Hexuronic acid									
chromatography	glcA	idA $glcA\pm$	glcA idA±	glcA idA±	idA $glcA\pm$	glcA idA	glcA idA±	idA $glcA\pm$	$glcA\pm$ idA±
Carbazole-sulfuric / Hexosamine	1.17	0.48	1.03	1.25	0.51	0.75	1.48	0.47	0.71
Carbazole-borosulfuric / Hexosamine	1.14	0.89	1.03	1.13	0.90	0.96	1.27	0.91	0.91
Protein									
Nitrogen/hexosamine	1.32	1.35	1.29	1.72	1.44	1.56	2.78	1.28	—

* Isolated from 83 g of human dermis.

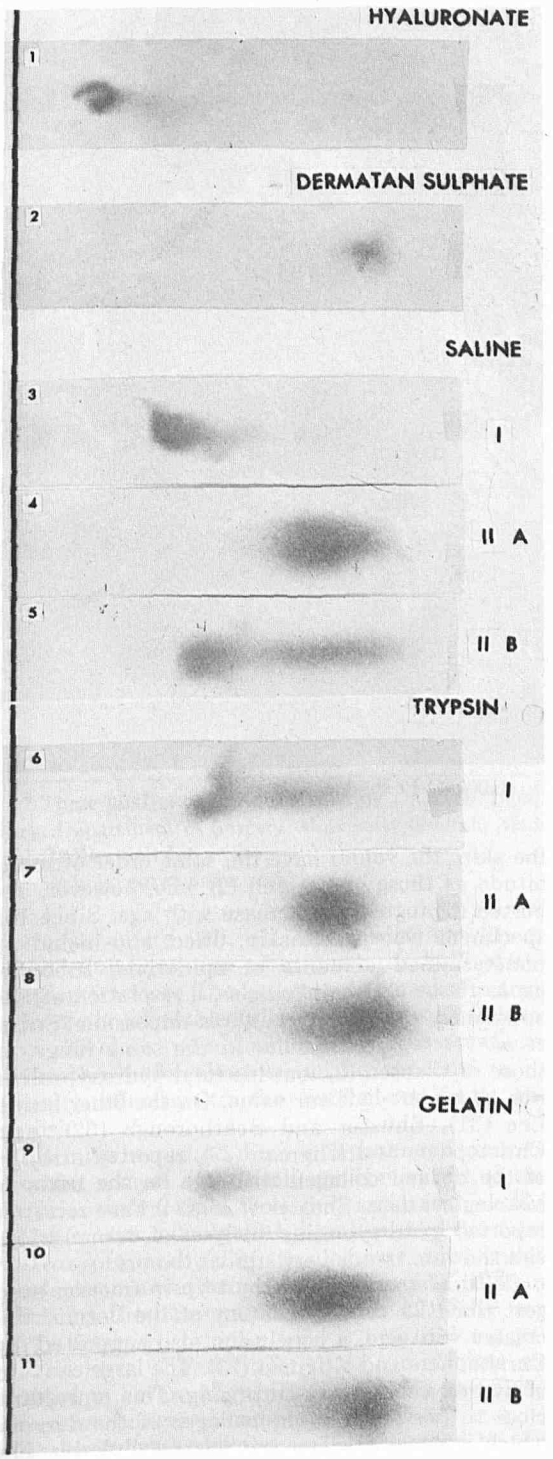

FIG. 10. *Electrophoreses of glycosaminoglycan fractions.*

tion IIA predominated and appeared to be dermatan sulfate; Fraction I resembled hyaluronate contaminated with dermatan sulfate; Fraction IIB, a hybrid of dermatan and chondroitin sulfates.

The Nature of the Residue

Histological. The residue was faintly eosinophilic, resistant to azan, and stained intensely with aldehyde fuchsin; thus it resembled elastin free of contaminating collagen and keratin (see Fig. 3). On swelling with water, homogenization and sonication, six shadowed preparations showed masses of fine fibrils strongly reminiscent of the similarly-prepared elastin of ligamentum nuchae and aorta (23) (see Fig. 11).

Chemical. A variety of analyses suggested that the residue was highly-purified elastin. A portion of each of the twenty-seven residues was hydrolyzed and the hydrolyzate was analyzed for nitrogen and hydroxyproline. The nitrogen content averaged 12.34 ± 0.64 (S.D.) mg-atoms per g, a value not differing significantly from the value of 11.89 ± 0.20 mg-atoms per g, calculated from reported analyses (23). The hydroxyproline content averaged 0.136 ± 0.029 (S.D.) mmole per g, compared with the value of 0.126 ± 0.013 mmole per g, also calculated from Gotte *et al.* (23). Elastin is the only mammalian protein other than collagen to contain hydroxyproline (24, 25). The hydroxyproline content of elastin is less than one-tenth that of collagen. Thus, the slight excess hydroxyproline in the dermal elastin, when compared with elastin derived from ligamentum nuchae and aorta, suggests that the maximal possible contamination of the residue with collagen was one percent. Six samples, selected to represent diverse ages and sexes were subjected to amino acid analysis with the results shown in Table VI. The recovery of nitrogen ranged from 85 to 112 percent, averaging 95 percent.

Effect of age. The weight of the residue increased steadily with age from a value close to 6 mg per g fresh wt at 20 years to 17 mg per g fresh wt at 80 years (Fig. 12).

DISCUSSION

Specimens. The selection of a group of patients *post-mortem* to represent a control population is a difficult task, particularly when both sexes and a diversity of ages are required. Accidental deaths seemed likely to provide a convenient group but these were not available in sufficient numbers. Of the group used in this study, those with chronic heart disease (cases 1, 8 and 11) and with tumors (cases 2 and 5) seemed least satisfactory. Since the latter groups showed no systematic differences from the other individuals studied, there seemed to be no grounds for excluding them from the series.

The region of the skin chosen for sampling was picked after much consideration and experimentation. The relative freedom from adnexal glands, the absence of rete pegs in the epidermis and the possibility of collecting reasonably generous samples all supported the choice. The results suggest that careful dissection of subcutaneous tissue and the use of homogeneous, dense connective tissue

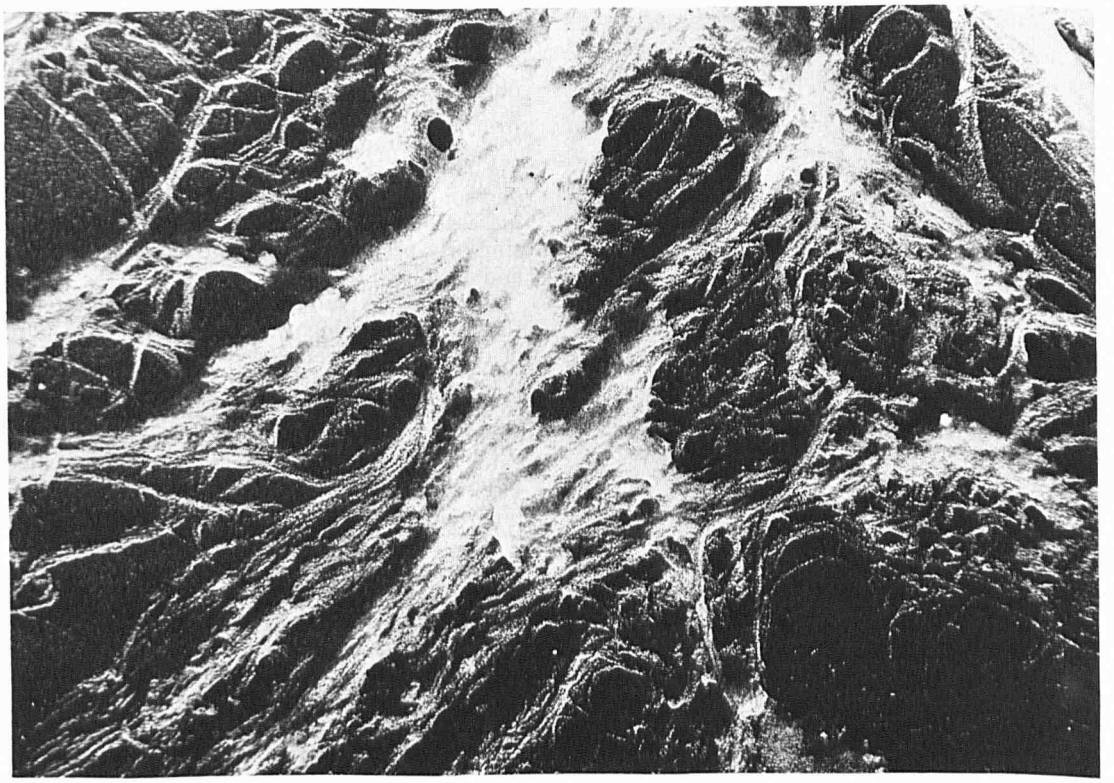

FIG. 11. *Sonicated residue* × 45,000, C-Pt shadow.

for analyses revealed trends which might otherwise have been obscured.

Anatomical significance of the fractions. The histological studies supported the 'chemical dissection' claimed for the extraction process (6). Prior to saline extraction, the cells and fibers of the tissue appeared to be intact. Apart from the separation of the individual collagen fibers of the bundle, the extraction with isotonic saline produced no gross histological change in the tissue. The treatment with trypsin removed essentially all evidence of cellular content in the tissue. Although the work of Drake *et al.* (26) suggests further that telopeptides derived from the action of trypsin on insoluble collagen should also be present, the respective contributions of insoluble collagen and cells to the tryptic digest could not be assessed rigorously. Subsequent studies, using sodium dodecyl sulfate rather than trypsin to remove the cells, have given comparable analyses (27). If it is assumed that the detergent had a minimal solubilizing effect on the insoluble collagen, the trypsin fraction may be taken as representing the cells of the dermis. Autoclaving removed all histological evidence of collagen. Hydroxyproline analyses of the residue indicated that it contained less than one percent of collagen. Thus, the gelatin fraction has been taken to represent the collagen of the dermis.

Collagen. The progressive decline in the collagen content of the dermis with age was surprising. When correction is made for the water of the skin, the values have the same order of magnitude as those of Clausen (2) who, however, reported a progressive increase with age. Since his specimens were whole skin, dried, and including undetermined amounts of epidermis, subcutaneous tissue and hair follicles, a resolution of the apparently conflicting results is impossible. Sobel *et al.* (1) reported results in the same range as those of Clausen (2), but his total hydroxyproline was about one-half our value. On the other hand, Lee (31), Shuster and Scarborough (32), and Christophers and Kligman (33), reported atrophy of the dermal collagen with age on the basis of histological data. Shuster *et al.* (34) have recently reported hydroxyproline analyses of dermal biopsies showing trends very similar to ours.

Cells. The analyses of the trypsin fraction suggest that the cellular content of the dermis decreases with age, a conclusion also supported by Christophers and Kligman (33). The large content of hydroxyproline was surprising. This represents close to one-tenth of the collagen of the dermis, which is considered to occur extracellularly. The amounts found in the trypsin fraction seem too large to represent intracellular material in transit from the site of synthesis to its usual extracellular locus. On the other hand, the prior exhaustive treatment of the tissue with isotonic saline, the demonstrated resistance of the hydroxyproline- and hexosamine-containing moieties to solution in the buffer (6), and the essentially identical composition of extracts prepared with sodium

TABLE VI

*Amino acid analyses of the residue**

Amino Acid	Residues/1000 residues in specimen no.					
	1	3	5	6	10	13
Hypro	15	12	11	12	16	23
Asp	6.7	5.1	4.6	4.5	7.8	6.2
Thr	14	12	14	10	13	15
Ser	9.9	8.3	8.8	7.6	9.8	9.1
Glu	17	19	16	18	21	20
Pro	130	152	116	129	135	133
Gly	303	291	306	304	314	276
Ala	221	220	221	228	204	231
Val	138	135	148	144	134	138
Ile	26	24	25	24	23	23
Leu	59	61	59	57	60	55
Tyr	21	21	23	21	23	22
Phe	24	22	22	22	23	20
Ides**	1.2	1.0	2.1	1.1	1.1	1.0
Des**	1.4	1.7	2.1	1.4	1.4	1.4
X3	1.0	*tr*	—	3.0	0.7	0.3
X4	1.0	0.8	0.2	0.8	0.7	0.6
Lys	11	5.0	5.1	5.0	6.8	7.4
Arg	—	8.3	15	7.5	9.6	18
Total	1000.2	999.2	998.9	999.9	1003.9	1000.0
Recovery (%N)	90	85	112	92	92	98

* These analyses were performed by Dr. L. B. Sandberg, Department of Surgery, University of Utah, Medical Center, Salt Lake City.

** Whole, not quarter, residues.

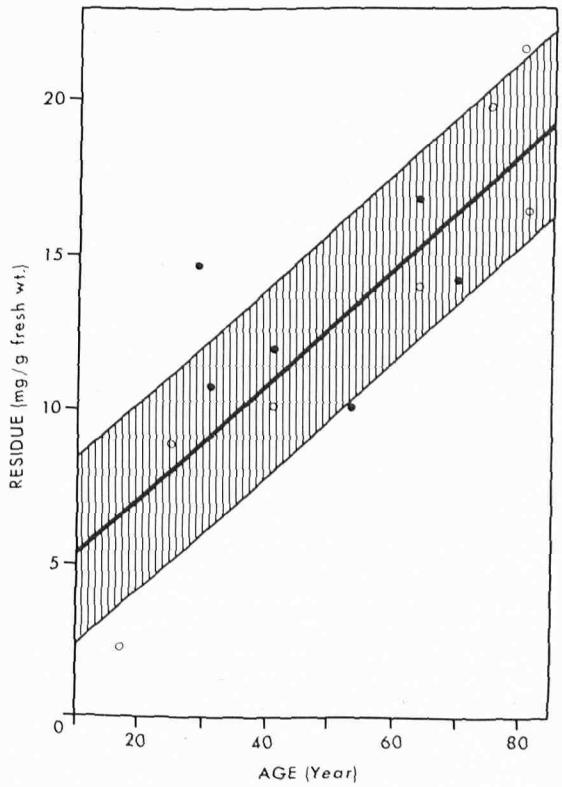

FIG. 12. *The weight of the residue* in men (●) and women (○).

dodecyl sulfate (27), all are consistent with an intracellular location of at least the bulk of the trypsin fraction. The possible dispersal of a fraction of the insoluble collagen by lyotropic relaxation (35) does not account for these observations, since the buffer alone would be expected to be equally effective to the buffer containing the trypsin.

Changes with age. Four components appear to account almost completely for the weight of the dermis: water, lipid, collagen, and residue. The collagen was calculated by multiplying the total hydroxyproline by 6.94, the factor recommended by Jackson and Cleary (25). The sum of the four fractions then amounted to 932 ± 27 (S.D.) mg per g fresh wt, ranging from 878 to 986 mg per g. The lines of best fit for these components are plotted in Figure 13, which is intended to convey the gross changes in composition with age.

A measurement of the area represented by the specimen at the time of collection would have assisted greatly in the interpretation of these results. The apparent increase in water may represent a relative change only, due to the atrophy of the collagen.

Plasma proteins. Earlier workers have demonstrated extravascular plasma proteins in the skin of the rabbit (36), cattle (37), rat (6, 38–40) and man (41, 42). Each of these groups agree that the

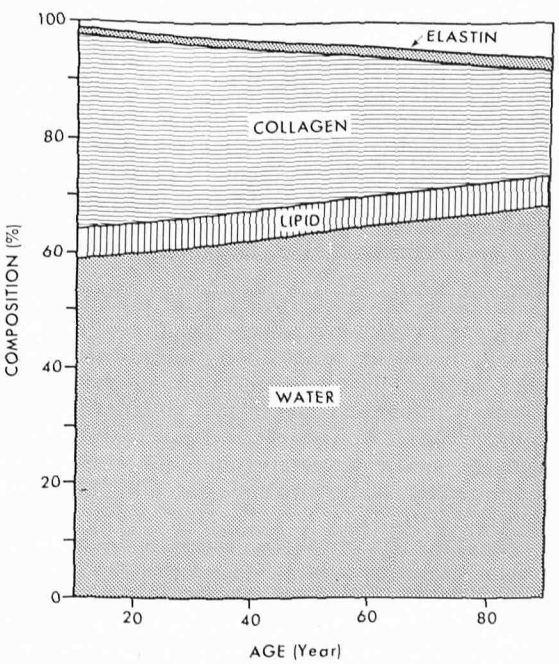

FIG. 13. *Age and the constitution of human dermis.* The lines of best fit to the analytical data were calculated for the major constituents.

bulk of the non-collagenous protein of the dermis derives from the proteins of the plasma. Opinion differs regarding the relative proportions of the individual proteins of the dermis relative to plasma and regarding the presence of proteins other than those derived from plasma. This present study was not designed to resolve these differences but suggests that at least one major non-plasma protein was present, in agreement with Fleischmajer and Krol (42), and that the proportions were similar to those of the plasma.

Glycosaminoglycans. The major glycosaminoglycan of the ground substance was hyaluronate, as previously reported for rat skin (43). This polysaccharide contributed about 25 percent of the hexosamine of the dermis, confirming the now generally-accepted conclusion that hexosamine is a meaningless measure of the 'ground substance', 'gel', or glycosaminoglycan content of the dermis (8, 22, 44). The hyaluronate content did not decrease with age (8). This finding is not contrary to the reported high levels of glycosaminoglycan, particularly of hyaluronate, reported in the skin of embryos and children (45). It does, however, disagree with the data of Clausen (2, 3) and Loewi (46); the former conducted much less extensive fractionation but included many more specimens so may have detected trends not apparent with so few specimens; the latter processed only a few pooled specimens. The absence of the epidermis from our specimens, a portion of the skin known to contain a considerable fraction of the total glycosaminoglycan (22), may also account for the discrepancy.

Thus, our data offer no support for the frequent claim that the 'ground substance' diminishes with age (1, 2). However, the increase in water and decrease in collagen content with age suggested an increased volume of water may be available for dissolved constituents. This change could permit a transformation of the interstitial space from a gel to a sol; or, if the 'ground substance' is not actually a gel, a progressive loss in its viscosity with age. Such relationships would account for the accelerated sodium chloride clearance from aged skin (47).

The association of a fraction of the dermatan sulfate with insoluble collagen confirms data previously noted for rat skin (48) and provides further support for the concept of an association between insoluble collagen and sulfated glycosaminoglycans (49). The interaction between soluble collagen and a dermatan sulfate-protein isolated from dermis has been demonstrated by Toole and Lowther (50).

Residue. The residue appeared to be homogeneous elastin on several grounds: the appearance in the light microscope; the affinity for common stains; and the ultrastructure apparent after sonication. The amino acid analyses corresponded closely to recently published analyses of elastin (51–55): proline, glycine, alanine and valine together comprised 78 to 81 percent of the residues; desmosine, isodesmosine, lysinonorleucine (X3) and X4 were present; leucine and isoleucine were present; threonine occurred in amounts exceeding serine.‡ The hydroxyproline and nitrogen analyses also supported this conclusion.

The average elastin content of the dermis increased nearly three-fold between the ages of 20 and 80 years. At least part of this effect can be attributed to the loss of insoluble collagen. Although other workers agree that the relative content of elastin increases with age (33), atrophy of collagen of a degree sufficient to account for these results seems unlikely to have been overlooked by either gross or light-microscopic observation. The changes noted in sun-damaged skin seem likely to be an exacerbation of the normal process of aging (56).

Equipment was supplied in part by the B. C. Medical Research Foundation. The collection of specimens was done by the autopsy room staffs of the Departments of Laboratories of the Vancouver General and St. Paul's Hospital and the Coroner's Office, City of Vancouver. Amino acid analyses were performed through the kindness of Dr. L. B. Sandberg, Department of Surgery, University of Utah Medical Center, Salt Lake City, Utah 84112, U. S. A.

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